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(54) Title: USE OF CYCLOSPORIN A 7-THIOAMIDE DERIVATIVES FOR HAIR GROWTH

(57) Abstract: The present invention discloses a composition comprising a cyclosporin A derivative having an excellent hair revitalizing activity as an active ingredient, and more particularly, a composition comprising cyclosporin A 7-thioamide produced by chemical derivatization of cyclosporin A as an active ingredient for promoting hair growth.

USE OF CYCLOSPORIN A 7-THIOAMIDE DERIVATIVES FOR HAIR GROWTH

Technical Field

The present invention relates to a hair growth promoter comprising a cyclosporin derivative as an active ingredient. More particularly, the present invention relates to a hair growth promoter comprising cyclosporin A 7-thioamide produced by chemical derivation of cyclosporin A as an active ingredient.

Background Art

On average, the human scalp contains about 100,000 to 150,000 hairs. Each hair has three main stages of growth: anagen, catagen and telogen, after which the hair falls out. This hair growth cycle is repetitive and the duration of one cycle is different from other cycles, ranging approximately 3 to 6 years. Thus, the average adult normally loses about 50 to 100 hairs every day. In general, alopecia refers to a phenomenon wherein duration of the anagen growth phase is shortened and the percentage of hairs in the catagen and telogen phases increases, whereby the number of lost hairs is increased excessively and abnormally.

There are many theories to explain for loss of hair, including for example, poor blood circulation, excessive functioning of male sex hormone, excessive production and secretion of sebum, deterioration of scalp by peroxides, bacteria, etc., hereditary factors, aging, stress, etc. However, explicit mechanisms have not been revealed. Recently, the population suffering from hair loss is tending to increase, since changing dietary habits and stress imposed on individuals due to

modern social environments, etc. has increased. Also, the age of the individuals affected by alopecia is dropping and furthermore, the population of female alopecia sufferers is rising.

One of preparations which are most commonly used for treatment and prevention of alopecia is one that contains minoxidil. There are two hair-regrowth agents which have received approval from the U.S. Food and Drug Administration, and minoxidil is one of those approved hair-regrowth agents. Minoxidil was originally developed as a hypertension drug for the purpose of reducing blood pressure. However, when using this drug, as a side effect, a trichogenous effect was observed and thereafter, this drug became famous as a hair-regrowth agent. Although mechanisms by which minoxidil works as a hair-regrowth agent is not clearly understood, it is inferred that minoxidil increases blood flow by expansion of blood vessels, whereby roots of hairs are supplied with more nutrition and eventually, growth of hairs are promoted.

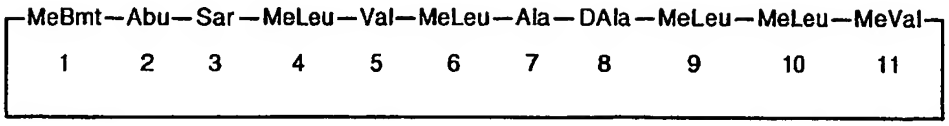
Such a model of blood flow increase has been indirectly supported by a recent report that minoxidil enhances the expression of vascular endothelial growth factor (VEGF), a growth factor associated with vasodilatation in the dermal papilla which is a main cell making up the hair roots (Br. J. of Dermatol., 1998, 138:407-411). Also, other than the vasodilative effect of the minoxidil in the hair-restoring mechanism, it has been reported that minoxidil promotes activation of dermal papilla cells in the roots of hair incubated *in vitro*, and growth of hair follicles in a tissue culture of follicles *in vitro* (Skin Pharmacol., 1996, 9:3-8 and J. Invest. Dermatol., 1989, 92:315-320). These

facts indicate that minoxidil may work directly on the roots of hair as a growth factor.

In addition, finasteride, a main component of Propecia which has started to be sold by Merck, is used for treatment of alopecia. It inhibits conversion of the male hormone testosterone into dihydrotestosterone, which is a more potent male hormone than testosterone. On December of 1997, the 1 mg finasteride tablet was approved by the US FDA as a hair-regrowth agent for treatment of male pattern hair loss in men only, and is now commercially available. In clinical studies, it has been demonstrated to have a significant trichogenous effect. However, there has been a report that finasteride may inhibit male sexual function as a side effect (J. Am. Acad Dermatol., 1998, 39:578-589). Since neither finasteride nor minoxidil show superior effect in clinical tests, and there is concern about side effects, many researches are conducted to develop a new and improved hair-regrowth agents.

The cyclosporin family of drugs has immunosuppressive activity. It is also effective to inhibit growth of virus, fungus, protozoan, etc. and has various physiological effects such as neoprotoxicity, hepatotoxicity, hypertension, enlargement of periodontium, trichogenous effect, and so on, as side effects (Advances in Pharmacol., 1996, 35:114-246 and Drug Safety, 1994, 10:310-317). Cyclosporin A, a representative cyclosporin, is a cyclic peptide having the following Chemical Formula, which comprises 11 amino acids, including several N-methyl amino acids and D-alanine at No. 8 residue.

[Structure Formula 1]



where MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine, Abu is L-α-aminobutyric acid, Sar is sarcosine, MeLeu is N-methyl-L-leucine,Val is L-valine, Ala is L-alanine, DAla is D-alanine, MeVal is N-methyl-L-valine.

The amino acid form of cyclosporin A of the above Chemical Formula 1 is L-configuration, unless otherwise specified. The residue numbering of amino acids starts from MeBmt and proceeds clockwise, i.e. 1 for MeBmt and 11 for the last MeVal (N-methyl-L-valine) as shown in the Structure Formula 1. Nomenclature of various derivatives including cyclosporins A to Z, follows methods commonly used (Helv. Chim. Acta, 1987, 70:13-36). For example, cyclosporins B and C, in which only L-α aminobutyric acid, No. 2 residue of cyclosporin A, is substituted with L-alanine and L-threonine, respectively, are expressed by describing the different residues and the positions thereof, that is $[Ala]^2$ cyclosporin and $[Thr]^2$ cyclosporin.

Thioamide derivatives of cyclosporin, in which the carbonyl oxygen (O) of amino acid(s) of either No. 4 or No. 7 residue, or both is substituted with sulfur (S) are named as cyclosporin 4-thioamide ($[^4\psi^5$ CS-NH] cyclosporin), cyclosporin 7-thioamide ($[^7\psi^8$ CS-NH] cyclosporin), and cyclosporin 4,7-bis(thioamide) ($[^7\psi^8$ CS-NH; $^4\psi^5$ CS-NH] cyclosporin), according to known methods (Helv. Chim. Acta 1991, 74:1953-1990; J. Org. Chem. 1993, 58:673-677; and J. Org. Chem. 1994, 59:7249-7258).

So far, possible development of cyclosporin as a

hair-regrowth agent has been studied by many research groups. Particularly, researches involving animal hair regrowth tests (Arch, Dermatol. Res., 1996, 288:408-410), human alopecia areata (J. Am. Acad. Dermatol., 1990, 22:242-250), human male pattern alopecia (J. Am. Acad. Dermatol., 1990, 22:251-253 and Skin Pharmacol., 1994, 7:101-104), and inhibition effect of hair loss by chemotherapy in animal models (Clin. Lab. Invest., 1995, 190:192-196 and Am. J. Pathol., 1997, 150:1433-1441) have been widely conducted. In comparative experiments on mouse's back, it is shown that cyclosporin has a hair regrowth effect about 100 times superior to minoxidil Based on such findings, there have been attempts to utilize cyclosporin as a treatment for male pattern alopecia, and many applications for patents have been filed.

For example, Japanese Patent Publication Kokai Nos. Sho 60-243008, Sho 62-19512 and Sho 62-19513 disclose use of cyclosporin derivatives as a hair regrowth agent. Also, European Patent Publication No. 0414632 B1 discloses a cyclosporin derivatives with modified No. 8 residue, PCT Patent Publication No. WO 93/17039 and PCT Patent Publication No. WO 00/51558 disclose isocyclosporin and immunosuppressive cyclosporin derivatives, respectively. These cyclosporins and derivatives thereof are provided as a hair regrowth agent. Furthermore, in U.S. Patent No. 5,807,820 and U.K. Patent No. 2,218,334 A, preparations containing cyclosporins with excellent transdermal absorption are suggested for new application of a hair regrowth agent.

However, there are still demands for a novel hair regrowth agent with superior hair restoring activity and

no side effects.

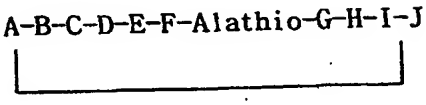
Disclosure of the Invention

Therefore, the present invention has been made in view of the above problems, and it is an object of the present invention to provide a novel hair growth promoter having hair regrowth activity and selected from thioamide derivatives of cyclosporin having carbonyl oxygen (O) of either of amino acid(s) No. 4 or No. 7, or both substituted with sulfur (S). The thioamide derivatives of cyclosporin substituted with sulfur have been used for studies of various derivations of cyclosporin molecules (Helv. Chim. Acta 1991, 74:1953-1990, J. Org. Chem. 1993, 58:673-677 and J. Org. Chem. 1994, 59:7249-7258). The present inventors has synthesized three thioamide derivatives of cyclosporin: cyclosporin 7-thioamide ([$^{7}\psi^{8}$ CS-NH] cyclosporin), in which the carbonyl oxygen (O) of amino acid No. 4 in the cyclosporin molecule is substituted with sulfur (S), cyclosporin 4-thioamide ([$^{4}\psi^{5}$ CS-NH] cyclosporin), in which the carbonyl oxygen (O) of amino acid No. 7 in the cyclosporin molecule is substituted with sulfur (S), and cyclosporin 4,7-bis(tioamide)([$^{7}\psi^{8}$ CS-NH; $^{4}\psi^{5}$ CS-NH] cyclosporin, in which the carbonyl oxygens (O) of amino acids Nos. 4 and 7 are substituted with sulfur (S), and examined for their hair regrowth effect. As a result, it was found that not all of those derivatives have hair regrowth effect, but only cyclosporin 7-thioamide ([$^{7}\psi^{8}$ CS-NH] cyclosporin, $C_{62}H_{111}N_{11}O_{11}S$) does have the hair regrowth effect.

Thus, the present invention is directed to, as a hair growth promoter, cyclosporin 7-thioamide ([$^{7}\psi^{8}$ CS-NH] cyclosporin, $C_{62}H_{111}N_{11}O_{11}S$) represented by the

Chemical Formula 1.

[Chemical Formula 1]



in which

A is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine (MeBmt), (2S,3R,4R,6E)-3-sulfhydryl-4-methyl-2-(methylamino)-6-octenoic acid, or (2S,4R,6E)-3-oxo-4-methyl-2-(methylamino)-6-octenoic acid;

B is L-α -aminobutyric acid (Abu), L-alanine (Ala), L-threonine (Thr), L-valine (Val), or L-norvaline (Nva);

C is sarcosine, D-methylalanine ((D)-$N(CH_3)$-$CH(CH_3)$-CO-), D-2-(methylamino)pent-4-enoyl ((D)-$N(CH_3)$-$CH(CH_2CHCH_2)$-CO-), (D)-2-(methylamino)pent-4-ynoyl ((D)-$N(CH_3)$-$CH(CH_2CCH)$-CO-), or D-methylthiosarcosine (D-Sar(2-Sme), (D)-$N(CH_3)$-$CH(SCH_3)$-CO-);

D is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-valine;

E is L-valine, or L-norvaline;

F is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine;

Alathio is L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-$CHCH_3$-CS-);

G is D-alanine, or D-serine;

H is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine;

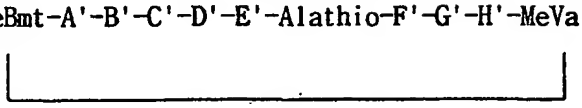
I is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine; and

J is N-methyl-L-valine or L-valine.

The preferred derivatives of cyclosporin of the above Chemical Formula 1 having hair regrowth activity are compounds represented by the following Chemical

Formula 2.

[Chemical Formula 2]



in which

MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine,

A' is L-α -aminobutyric acid (Abu), L-alanine (Ala), L-threonine (Thr), L-valine (Val), or L-norvaline (Nva);

B' is sarcosine, D-methylalanine ((D)-$N(CH_3)$-$CH(CH_3)$-CO-), D-2-(methylamino)pent-4-enoyl ((D)-$N(CH_3)$-$CH(CH_2CHCH_2)$-CO-), (D)-2-(methylamino)pent-4-ynoyl ((D)-$N(CH_3)$-$CH(CH_2CCH)$-CO-), or D-methylthiosarcosine (D-Sar(2-Sme), (D)-$N(CH_3)$-$CH(SCH_3)$-CO-);

C' is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-valine;

D' is L-valine, or L-norvaline;

E' is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine;

Alathio is L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-$CHCH_3$-CS-);

F' is D-alanine, or D-serine;

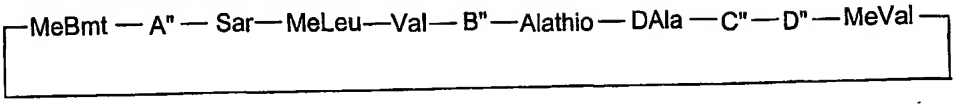
G' is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine;

H' is N-methyl-L-leucine, γ-hydroxy-N-methyl-L-leucine, or L-leucine; and

MeVal is N-methyl-L-valine.

The more preferred thioamide derivatives of cyclosporin of the above Chemical Formula 1 having hair regrowth activity are compounds represented by the following Chemical Formula 3.

[Chemical Formula 3]



in which

MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine,

A" is L-α -aminobutyric acid (Abu), L-alanine (Ala), L-threonine (Thr), L-valine (Val), or L-norvaline (Nva);

Sar is sarcosine;

MeLeu is N-methyl-L-leucine;

Val is L-valine;

B" is N-methyl-L-leucine, or L-leucine;

Alathio is L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-$CHCH_3$-CS-);

DAla is D-alanine;

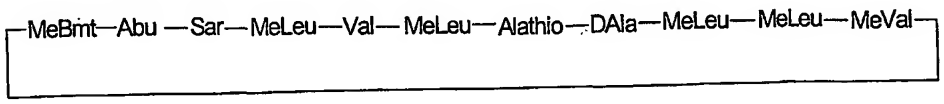
C" is N-methyl-L-leucine, or L-leucine;

D" is N-methyl-L-leucine, or L-leucine; and

MeVal is N-methyl-L-valine.

The even more preferred thioamide derivatives of cyclosporin of the above Chemical Formula 1 having hair regrowth activity are compounds represented by the following Chemical Formula 4.

[Chemical Formula 4]



in which

MeBmt is N-methyl-(4R)-4-[(E)-2-butenyl]-4-methyl-L-threonine;

Abu is L-α -aminobutyric acid;

Sar is sarcosine;

MeLeu is N-methyl-L-leucine;

Val is L-valine;

Alathio is L-alanine thioamide ([$^{7}\psi^{8}$ CS-NH], NH-$CHCH_3$-CS-);

DAla is D-alanine; and

MeVal is N-methyl-L-valine.

The cyclosporin A 7-thioamide is a derivative of cyclosporin A, in which the carbonyl oxygen (O) of amino acid No. 7 in the cyclosporin A molecule is substituted with sulfur (S), that is, [$^{7}\psi^{8}$ CS-NH](NH-$CHCH_3$-CS-)cyclosporin A ($C_{62}H_{111}N_{11}O_{11}S$).

In another aspect, the present invention is directed to a hair growth promoter formulated into a liquid formulation, spray, gel, paste, emulsion, cream, conditioner, or shampoo.

Brief Description of the Drawings

The above and other objects, features and advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which:

Fig. 1 is a ^{1}H-NMR spectrum of cyclosporin A 7-thioamide;

Fig. 2 is a ^{13}C-NMR spectrum of cyclosporin A 7-thioamide;

Fig. 3 is a result of the TOCSY (Total Correlation Spectroscopy) of cyclosporin A 7-thioamide;

Fig. 4 is a photograph of a control group in the animal test measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice;

Fig. 5 is a photograph of a group treated with cyclosporin A in the animal test measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice;

Fig. 6 is a photograph of a group treated with cyclosporin A 7-thioamide in the animal test measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice;

Fig. 7 is a photograph of a group treated with cyclosporin A 4,7-bis(thioamide) in the animal test measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice; and

Fig. 8 is a photograph of a group treated with cyclosporin A 4-thioamide in the animal test measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice.

Best mode for carrying out the invention

The present invention is described in detail as follows. In order to develop a novel hair regrowing agent, the present inventors synthesized various derivatives of cyclosporin A and carried out the hair regrowth evaluation tests for the synthesized derivatives. As a result, it was found that cyclosporin A 7-thioamide has an superior hair regrowth (restoring) effect to any other compounds.

The following Reference Example and Examples are given by way of illustration of the best mode contemplated by the inventor(s) of carrying out the invention. However, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention.

Reference Example 1

Step 1: synthesis of acetylcyclosporin A

2.4 g (2.0 mmol) of cyclosporin A and 0.24 g (2.0

mmol) of 4-(dimethylamino)pyridine were added to 20 ml of pyridine and 20 ml of acetic anhydride, stirred for 18 hours at room temperature, and distilled under reduced pressure. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$). The crude product was purified by chromatography on a silica gel column to give 2.3 g of the title compound.

Step 2-1: synthesis of acetylcyclosporin A 4,7-bis(thioamide)

1.8 g (1.45 mmol) of acetylcyclosporin A was dissolved in 50 ml of xylene. The resulting solution was heated to 130°C and 0.35 g (0.87 mmol) of Lawesson reagent was added in small amounts thereto. The reaction solution was stirred for 30 minutes at 130°C, cooled to room temperature, and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column to give 0.57 g of the title compound.

Step 2-2: synthesis of acetylcyclosporin A 4-thioamide

1.8 g (1.45 mmol) of acetylcyclosporin was dissolved in 50 ml of xylene. The resulting solution was heated to 130°C and 0.35 g (0.87 mmol) of Lawesson reagent was added in small amounts thereto. The reaction solution was stirred for 30 minutes at 130°C, cooled to room temperature, and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to

form a crude product. The crude product was purified by chromatography on a silica gel column to give 0.08 g of an acetoxy compound, i.e. the title compound.

Step 3-1: synthesis of cyclosporin A 4,7-bis(thioamide)

0.32 g (0.25 mmol) of acetylcyclosporin A 4,7-bis(thioamide), an acetoxy compound, was dissolved in 50 ml of methanol, and 20 ml (10 mmol) of 0.5M sodium methoxide (NaOMe) in methanol was added thereto, followed by stirring for 4 hours at room temperature. The reaction solution was neutralized using acetic acid and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column and then HPLC to give 0.27 g of the title compound.

Step 3-2: synthesis of cyclosporin A 4-thioamide

0.2 g of acetylcyclosporin A 4-thioamide, an acetoxy compound, was dissolved in 50 ml of methanol, and 20 ml (10 mmol) of 0.5M sodium methoxide (NaOMe) in methanol was added thereto, followed by stirring for 4 hours at room temperature. The reaction solution was neutralized using acetic acid and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column and then HPLC to give 0.16 g of the title compound.

Example 1

Synthesis of cyclosporin A 7-thioamide

Step 1: synthesis of acetylcyclosporin A

2.4 g (2.0 mmol) of cyclosporin A and 0.24 g (2.0 mmol) of 4-(dimethylamino)pyridine were added to 20 ml of pyridine and 20 ml of acetic anhydride, stirred for 18 hours at room temperature, and distilled under reduced pressure. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$). The crude product was purified by chromatography on a silica gel column to give 2.3 g of the title compound.

Step 2: synthesis of acetylcyclosporin A 7-thioamide

1.8 g (1.45 mmol) of acetylcyclosporin A was dissolved in 50 ml of xylene. The resulting solution was heated to 130°C and 0.35 g (0.87 mmol) of Lawesson reagent was added in small amounts thereto. The reaction solution was stirred for 30 minutes at 130°C and cooled to room temperature. The solution was then distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column to give 0.19 g of an acetoxy compound, i.e. the title compound.

Step 3: synthesis of cyclosporin A 7-thioamide

0.2 g of acetylcyclosporin A 7-thioamide, an acetoxy compound, was dissolved in 50 ml of methanol, and 20 ml (10 mmol) of 0.5M sodium methoxide (NaOMe) in methanol was added thereto, followed by stirring for 4 hours at room temperature. The reaction solution was

neutralized using acetic acid and distilled under reduced pressure to remove the solvent. To the residue was added 100 ml of methylene chloride. The residue was then washed with water and dried over dry magnesium sulfate ($MgSO_4$) to form a crude product. The crude product was purified by chromatography on a silica gel column and then HPLC to give 0.17 g of the title compound.

Confirmation of structure of cyclosporin A 7-thioamide

The molecular weight of cyclosporin A 7-thioamide prepared in Example 1 was determined using FAB MS (ZMS AX 505H). $[M+H]^+$ peak was observed at m/z 1218.8. Also, its structure was examined using ^{1}H-NMR (Bruker NMR 600 MHz) and ^{13}C-NMR (Bruker NMR 150 MHz). Chemical shift (δ) of hydrogens and carbons in alanine, No. 7 residue, where thioamide was expected to be formed, and upstream and downstream residues, i.e. N-methyl-L-leucine, No. 6 residue and D-alanine, No. 8 residue, was measured (Fig. 1 and Fig. 2). Based on the obtained data, the sequence of three residues was determined and compared with the structure of known molecules.

By TOCSY (total correlation spectroscopy), it was observed that α-protons in alanine 1 and alanine 2 give rise to chemical shifts of 4.73 ppm and 5.35 ppm, methyl hydrogens in R group give rise to chemical shifts of 1.58 ppm and 1.37 ppm, and there were 4 peaks for Leucine of No. 6 residue (Fig. 3). By COSY (Homonuclear correlated spectroscopy), amino hydrogens in alanine 1 and alanine 2 assigned 7.56 ppm and 8.86 ppm. By NOESY (Nuclear Overhauser effect spectroscopy), it was seen that 7.56 ppm of amino hydrogen in alanine 1 was located

near 4.95 ppm of α-proton, which turned out to be α-proton of leucine by TOCSY. Thus, partial connective sequence of alanine 1-leucine was determined. Meanwhile, in carbon NMR, a peak was observed at 202.15 ppm. That is, it is noted that when an oxygen atom in the carbonyl group was substituted with a sulfur atom, the peak of carbon moved toward downfield by about 30 ppm (downfield shift) and this chemical shift was caused by carbon of thioamide (Helv. Chim. Acta 1991, 74:1953-1990; J. Org. Chem. 1993, 58:673-677; and J. Org. Chem. 1994, 59:7249-7258). Further, by HMBC (Heteronuclear multiple bond correlation), based on the data that a peak was observed at 202.15 ppm and proximal hydrogens existing within 2 to 3 bonds were α-protons (4.73 pm and 5.35 ppm), amino hydrogens (7.56 ppm and 8.86 ppm), and methyl hydrogen (1.58 ppm) in alanine 1 and alanine 2, it is concluded that thioamide is located at alanine 1, and the sequence of alanine 2 (No. 8 D-alanine), alanine 1 (alanine No. 7) and leucine (No. 6 methyl leucine) was confirmed. Carbon chemical shift of amino acids Nos. 7 and 8 was measured using HMQC (Heteronuclear multiple quantum coherence).

No. 7 alanine: 600 MHz ^{1}H NMR (CDCl3): δ 7.56 (NH), 4.73 (H-Cα) and 1.58 (3H-Cβ).

150 MHz ^{13}C-NMR: δ202.15 (C=S), 55.97 (Cα), 20.14 (Cβ)

No. 8 alanine: 600 MHz ^{1}H NMR (CDCl3): δ 8.86 (NH), 5.35 (H-Cα) and 1.37 (3H-Cβ).

150 MHz ^{13}C-NMR: δ172.67 (C=O), 55.12 (Cα), 16.37 (Cβ)

FORMULATIONS

FORMULATION 1:

Preparation of a hair revitalizing tonic containing cyclosporin A 7-thioamide

3 hair revitalizing tonics with different contents of cyclosporin A 7-thioamide as described in Table 1 below were prepared. Respective ingredients were mixed and agitated so that solid ingredients were dissolved to form the hair revitalizing tonics in the form of a homogenous solution. The resulting tonics were examined for the hair regrowth effect in animal models according to Test Example 1 described later. In the animal test, it was shown that Composition 1, which contains 0.1% cyclosporin A 7-thioamide, has hair regrowth effect comparable to a hair revitalizing tonic containing 0.1% cyclosporin A.

Table 1

Ingredients	Composition 1	Composition 2	Composition 3
Ethanol	40.0	40.0	40.0
Cyclosporin A 7-thioamide	0.1	1.0	8.0
Tocopherol acetate	0.1	0.1	0.1
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Tween 20	0.5	0.5	0.5
Fragrance	Prop. amount	Prop. amount	Prop. Amount
Color	Prop. amount	Prop. Amount	Prop. Amount
Water	q.s. to 100 wt%		

Formulation 2:

Preparation of a hair cream containing cyclosporin A 7-thioamide

3 hair creams with different contents of cyclosporin A 7-thioamide as described in Table 2 below

were prepared. Oil phase ingredients were mixed and heated to 80°C so that the ingredients formed a homogenous mixture. Separately, aqueous phase ingredients were mixed and heated to 80°C so that the ingredients formed a homogenous mixture. The prepared two mixtures of different phases at 80 ℃ were combined and emulsified. The resulting emulsion was then cooled to room temperature and fragrance and colorant were added thereto to form a hair cream. At this stage, water was added to make up the volume of the hair cream.

The resulting hair creams were examined for their hair regrowth effect in animal models according to Test Example 1 described later. In the animal test, it was shown that Composition 1 described in Table 2, which contains 0.1% cyclosporin A 7-thioamide, has hair regrowth effect comparable to a hair cream containing 0.1% cyclosporin A.

Table 2

Ingredients	Composition 1	Composition 2	Composition 3
Paraffin	5.0	5.0	5.0
Setostearylalcohol	5.5	5.5	5.5
Petrolatum	5.5	5.5	5.5
Glycerine -monostearate	3.0	3.0	3.0
Polyoxyethylene octyldodecylether	3.0	3.0	3.0
Propylparaben	0.3	0.3	0.3
Cyclosporin A 7-thioamide	0.1	1.0	8.0
Glycerin	7.0	7.0	7.0
Dipropyleneglycol	20.0	20.0	20.0

Polyethyleneglycol	5.0	5.0	5.0
Water	q.s. to 100 wt% without fragrance and colorant		
Fragrance	Prop. Amount	Prop. Amount	Prop. Amount
Colorant	Prop. Amount	Prop. Amount	Prop. Amount

Formulation 3:

Preparation of a shampoo containing cyclosporin A 7-thioamide

3 shampoos with different contents of cyclosporin A 7-thioamide as described in Table 3 below were prepared. Ingredients except for the fragrance, colorant and water were mixed and heated while being stirred so that the ingredients formed a homogenous mixture. The resulting mixture was then cooled to room temperature and fragrance and colorant were added thereto. Finally, water was added to make up the volume of the shampoo.

Table 3

Ingredients	Composition 1	Composition 2	Composition 3
Sodium POE laurylsulfuric acid (30 wt% aqueous solution)	40.0	40.0	40.0
Palm oil fatty acid Diethanolamide	3.0	3.0	3.0
propylene glycol	2.0	2.0	2.0
Methyl paraoxybenzoic acid	0.2	0.2	0.2
Ethanol	2.0	2.0	2.0

Cyclosporin A 7-thioamide	1.0	3.0	10.0
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Fragrance	Prop. Amount	Prop. Amount	Prop. Amount
Colorant	Prop. Amount	Prop. Amount	Prop. Amount
Water	q.s. to 100 wt%		

Formulation 4:

Preparation of a hair conditioner containing cyclosporin A 7-thioamide

3 hair conditioners with different contents of cyclosporin A 7-thioamide as described in Table 4 below were prepared. Oil phase ingredients were mixed and heated to 80°C so that the ingredients formed a homogenous mixture. Separately, aqueous phase ingredients were mixed and heated to 80°C so that the ingredients formed a homogenous mixture. The prepared two mixtures of different phases at 80 ℃ were combined and emulsified. The resulting emulsion was then cooled to room temperature and fragrance and colorant were added thereto to form a hair conditioner. Here, water was added to make up the volume of the hair conditioner.

Table 4

Ingredients	Composition 1	Composition 2	Composition 3
Cetanol	3.0	3.0	3.0
Self-emulsifiable Glycerol-monostearate	2.0	2.0	3.0
Squalene	10.0	10.0	10.0
Cyclosporin A 7-thioamide	1.0	5.0	10.0

Propylene glycol	2.0	2.0	2.0
Stearyldimethyl Benzylammonium chloride (25 wt% aqueous solution)	8.0	8.0	8.0
Methyl paraoxybenzoic acid	0.2	0.2	0.2
Salicylic acid	0.3	0.3	0.3
L-menthol	0.3	0.3	0.3
Water	q.s. to 100 wt%		
Fragrance	Prop.amount	Prop.amount	Prop.amount
Colorant	Prop.amount	Prop.amount	Prop.amount

Test Example 1

Test of hair regrowth effects of the 7-thioamide derivative of cyclosporin A

C57BL/6 mice (female), 42 ~ 49 days old, were used in this test.

First of all, mice were removed of hair on their backs using an electric shaver, and weighed. The mice were divided into several groups with weights equally distributed. After one day of adaptation, cyclosporin A, cyclosporin A 7-thioamide and a control were applied in a dose of 100 μl (0.1% w/v) over the hair removed area once a day per each individual for 30 days. The degree of hair growth was observed by naked eye and the back sides of the mice were photographed. Fig. 4 shows a photograph of a control group measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice. Fig. 5 shows a photograph of a group treated with cyclosporin A measuring hair growth effects of cyclosporin A and thioamide derivatives

thereof using C57BL/6 mice. Fig. 6 shows a photograph of a group treated with cyclosporin 7-thioamide measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice, in which it is noted that the result is comparable to that of cyclosporin A (Fig. 5). Figs. 7 and 8 show photographs of groups treated with cyclosporin 4,7-bis(thioamide) and cyclosporin 4-thioamide, respectively, measuring hair growth effects of cyclosporin A and thioamide derivatives thereof using C57BL/6 mice, in which no significant effect is observed.

Upon observing the back conditions of mice during the test period of 30 days, no peculiar skin irritations were observed in the control group and all treated groups.

Test Example 2

Test of Immunosuppression of cyclosporin A 7-thioamide

Cyclosporin A 7-thioamide according to the present invention was examined for its ability to inhibit the cell division in peripheral blood mononuclear cells (PBMC) treated with PHA (Phytohemagglutin), a cell division stimulant according to the MLR method (Mixed Lymphocyte Reaction method, J. Antibiotics, 1994, 47:208-215).

A group of cells (4 x 10^6/ml) treated with mitomycin C (30 μg/ml, 30 min.) as stimulant cells was mixed with an equal number of reaction cell group non-treated. The resulting mixture was incubated for 4 days. During the incubation, the mixture was treated with cyclosporin A and derivatives thereof including cyclosporin A 7-thioamide in serial dilutions from 10^{-6}

mole to 10^{-11} mole. After 4 days incubation, to the mixtures ^{3}H-thymidine was added and incubated for additional 16 hours. Then, the amount of thymidine introduced into the cells was measured (liquid scintillation counter) and IC_{50} (μg/ml) of respective cyclosporins were calculated.

As a result, IC_{50}(μg/ml) of cyclosporin A was 0.056, 0.052 and 0.023, while cyclosporin A 7-thioamide was 0.581, 0.601 and 0.453. Thus, it was noted that cyclosporin A 7-thioamide had lower immunosuppressive effect than cyclosporin A, which accorded with the data in the literature (Helv. Chim. Acta, 1991, 74:1953-1990).

Also, to examine the ability of cyclosporin A 7-thioamide to inhibit cell division against stimulation by PHA, to mononuclear cells (4 x 10^{6}/ml) which had been treated with PHA (10 μg/ml) were added cyclosporin A and derivatives thereof including cyclosporin A 7-thioamide in serial dilutions from 10^{-6} mole to 10^{-11} mole, followed by incubation for 3 days. Then, like in the MLR method, ^{3}H-thymidine was added to the cells, which were again incubated for additional 16 hours. After the incubation, IC_{50} (μg/ml) of respective cyclosporins were calculated. From the result, it was seen that cyclosporin A 7-thioamide had reduced immunosuppression activity compared to cyclosporin A.

In the hair regrowth agent according to the present invention, the administrated amount capable of promoting hair regrowth is 0.01 to 30%, preferably 0.1 to 10% based on total weight of composition.

Industrial Applicability

A hair growth promoter comprising cyclosporin A 7-

thioamide as an active ingredient according to the present invention has excellent hair growth promoting effect, leading the superior hair restoring effect while maintaining lower immunosuppression.

WHAT IS CLAIMED IS:

1. A hair growth promoter comprising cyclosporin A 7-thioamide ([$^{7}\psi^{8}$ CS-NH] cyclosporin A) as an active ingredient.

2. The hair growth promoter as set forth in claim 1, which is formulated in a form selected from the group consisting of liquid formulation, spray, gel, paste, emulsion, cream, conditioner, and shampoo.

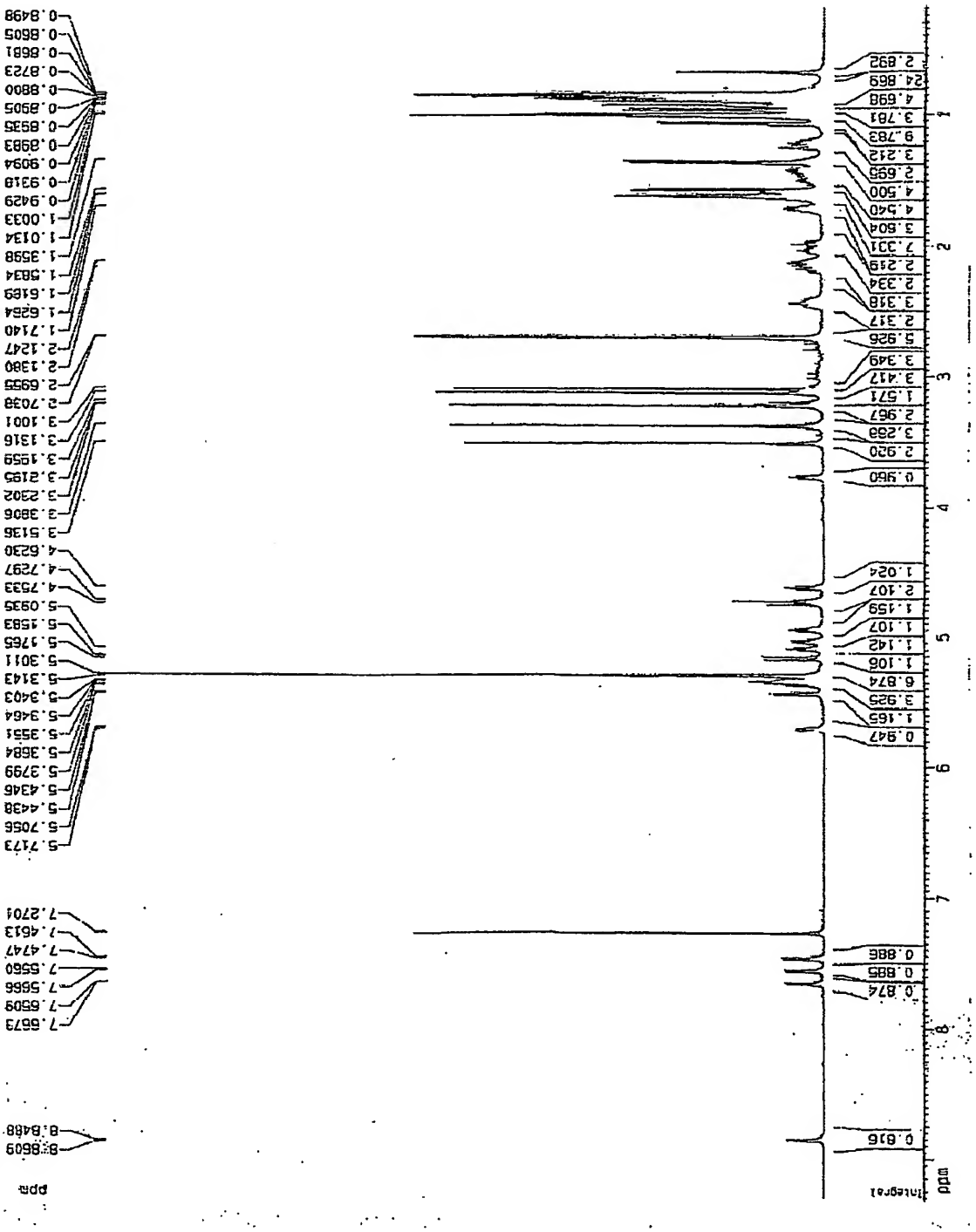


FIG. 1

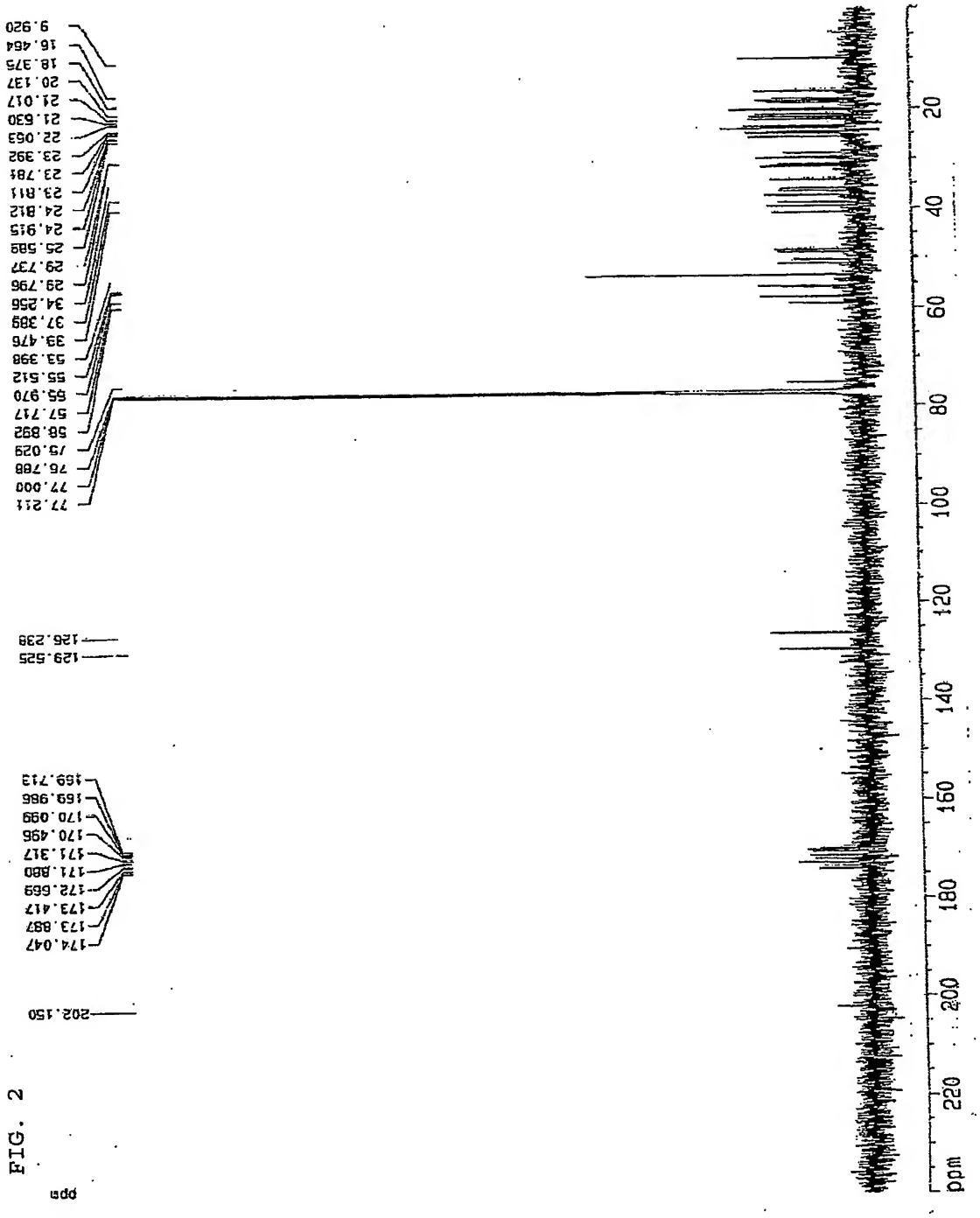

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FIG. 2

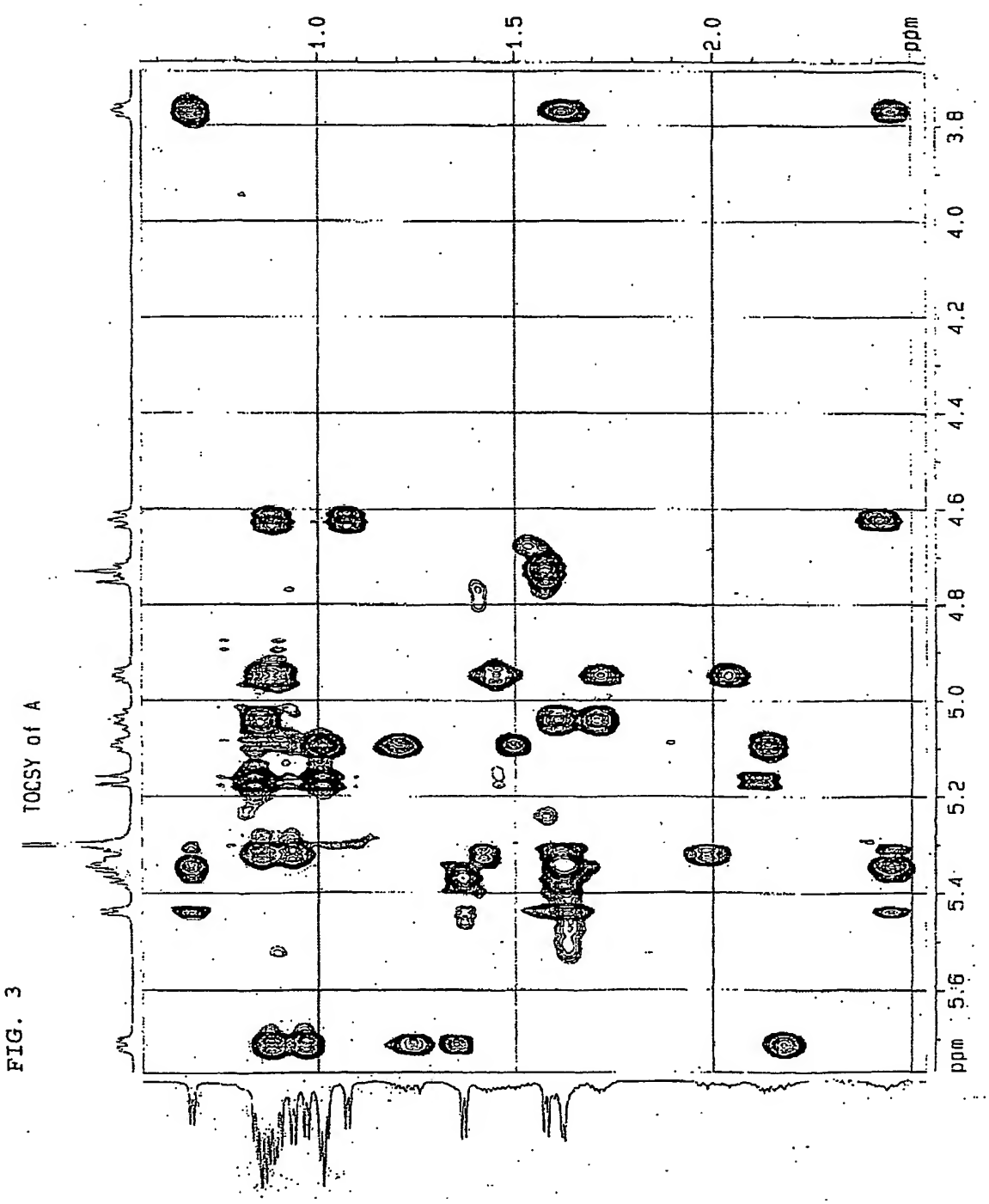

FIG. 3

FIG. 4

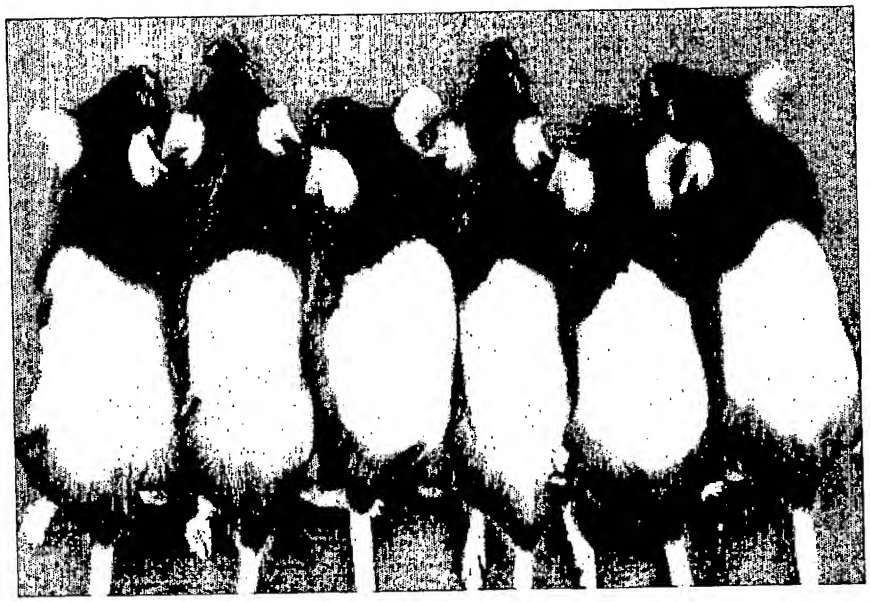

FIG. 5

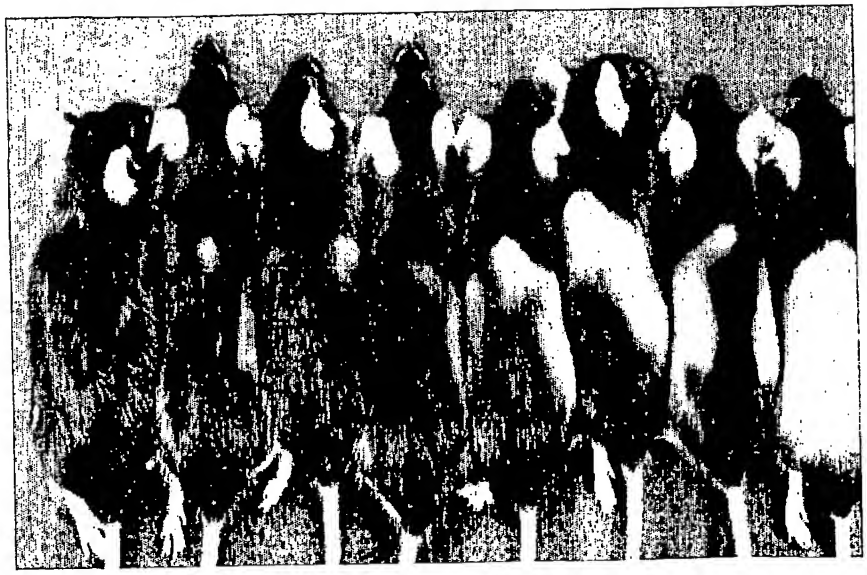

FIG. 6

FIG. 7

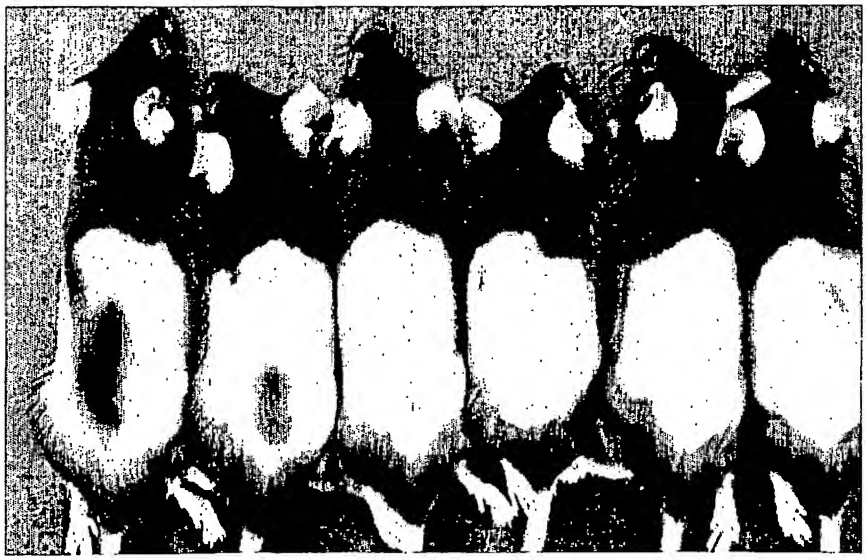

FIG. 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/01960

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 7/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K 7/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean Patents and Applications for Inventions since 1975

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAPLUS(STN), NPS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	MAURER et al., 'Hair growth modulation by topical immunophilin ligands: induction of anagen, inhibition of massive catagen development, and relative protection from chemotherapy-induced alopecia', Am. J. of Pathol., American Society for Investigative Pathology, US, 1997, 150(4), p1433-1441	1 - 2
A	YAMAMOTO et al., 'Hair growth-stimulating effects of cyclosporin A and FK506, potent immunosuppressants, J. Dermatol. Sci., 1994, 7(Suppl.), S47 - S54	1 - 2

☐ Further documents are listed in the continuation of Box C.

☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevence
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevence; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevence; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 MARCH 2002 (07.03.2002)	08 MARCH 2002 (08.03.2002)

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